MONOCLONAL ANTIBODIES TO GROUP II *DERMATOPHAGOIDES spp.* ALLERGENS: MURINE IMMUNE RESPONSE, EPITOPE ANALYSIS AND DEVELOPMENT OF A TWO SITE ELISA

Short title: Monoclonal ab to mite Group II allergens

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Supported by National Institutes of Health Grants AI-24687 and AI-24261.

This work was presented in part at the 48th Annual Meeting of the American Academy of Allergy and Clinical Immunology in Orlando, March, 1992.

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Abbreviations used:

Mab	Monoclonal antibody
BSA	Bovine serum albumin
RIA	Radioimmunoassay
ELISA	Enzyme-linked immunosorbent assay
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
HPLC	High-performance liquid chromatography
PBS-T	Phosphate-buffered saline, Ph 7.4, containing 0.05% Tween 20
BBS	Borate-buffered saline, Ph 8.0
SAS	Saturated ammonium sulfate

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ABSTRACT

The Group II allergens are a major cause of sensitization in mite allergic patients. To facilitate the antigenic analysis of Group II allergens and to develop improved methods of allergen detection, we compared IgG anti-Group II antibody responses in inbred mouse strains and raised a panel of monoclonal antibodies (Mab). IgG antibody responses were compared by antigen binding radioimmunoassay and the epitope specificity of the Mab was analyzed by two site binding assays and by cross-inhibition radioimmunoassays. Polyclonal IgG antibodies to Group II were directed against cross-reactive epitopes on *Der p* II and *Der f* II. Comparison of IgG antibody responses in 5 BALB congenic strains, showed that H-2^d mice were poor responders, whereas H-2^b or H-2^k mice made strong IgG anti-Group II responses. The specificity of 9 anti-*Der p* II IgG Mab raised in A/J mice was compared with 7 Mab produced previously. Most Mab (11/16) recognized cross-reactive epitopes on *Der p* II and *Der f* II: three were *Der p* II specific and two showed high binding to *Der f* II. Epitope analysis showed that the Mab defined four cross-reactive, non-overlapping sites on the Group II allergens. Binding of several combinations of mAb was compared and a two site enzyme immunoassay for Group II was developed. Linear regression analysis showed an excellent correlation between results of this assay and Group II radioimmunoassay of house dust samples ($n = 40$, $r = 0.85$, $p < 0.001$). The results show that there are multiple cross-reactive B cell epitopes on Group II allergens. The Group II ELISA has several important applications, including environmental allergen detection, monitoring intervention studies, and standardization of mite allergen extracts.

Key words: Monoclonal antibody, mite allergens, house dust, ELISA, *Dermatophagoides*

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INTRODUCTION

Immunochemical and molecular studies have defined four groups of allergens from *Dermatophagoides pteronyssinus* and *D. farinae*: Group I (25kd, cysteine proteases); Group II (14kd proteins); Group III (~30kd serine proteases); and Group IV (60kd, amylase). The Group I and Group II allergens are the most important cause of sensitization, inducing IgG and IgE antibody responses in 80-95% of mite allergic patients, and each of these allergens (*Der p* I, *Der f* I, *Der p* II and *Der f* II) has been cloned and sequenced (reviewed in 1). There is now strong evidence that sensitization and exposure to mite allergens is an important cause of asthma and an associated cause of atopic dermatitis (1-6). Much of this work has been based on clinical and epidemiologic studies using immunoassays for Group I allergens for environmental allergen detection. These assays have used either *Der p* I or *Der f* I specific monoclonal antibodies (mAb) or monospecific polyclonal antibodies in ELISA or RIA techniques (7-9). Assays for Group I allergens have also been used to assess the quantities and distribution of mite allergens in the air, and to assess the efficacy of physical and chemical mite avoidance procedures (1, 10-18).

Recent studies have compared the environmental distribution and airborne properties of Group II allergens (9, 12, 15, 19). However, measurements of Group II have not been widely adopted because relatively few anti-Group II mAb have been produced and it has only been possible to use the mAb in RIA procedures. The first anti-Group II mAb was raised against *Der p* II and, subsequently, three anti-*Der f* II mAb were produced (20, 21). These mAb recognized common epitopes on *Der p* II and *Der f*

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II and were used to develop affinity purification techniques and solid phase RIA's for Group II. Five other anti-*Der f* II mAb have also recently been produced (23).

The present paper compares the immune response to Group I and Group II allergens in inbred mouse strains and describes the production of a large panel of anti-*Der p* II mAb. Analysis of the epitope specificities of the mAb allowed a two-site ELISA to be developed. The results demonstrate differences between the magnitude and specificity of murine IgG antibody responses to mite allergens and that multiple non-overlapping epitopes on Group II allergens were defined by the mAb. The Group II ELISA showed a very good quantitative correlation with RIA. The results suggest that this assay will be useful in environmental studies, in assessing mite avoidance procedures, and for the standardization of mite allergen extracts.

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MATERIALS AND METHODS

Mite allergens

Group I and Group II allergens were purified from aqueous extract of *D. pteronyssinus* or *D. farinae* culture by mAb affinity chromatography (21). Immunosorbents were prepared using mAb 4C1, for *Der p* I and *Der f* I, and mAb αDpX or mAb 6D6 for *Der p* II and *Der f* II, respectively. Allergens were eluted with 0.005M glycine in 50% ethylene glycol, pH 10.0 (21).

Mouse immunizations

i) Comparison of IgG antibody responses to *Der p* I or *Der p* II.

Groups of 5 animals of six different mouse strains (BALB/c, C57Bl6, C3H, CBA, A/J and AKR) were immunized intraperitoneally, 3 times at 14 day intervals, with 10μg *Der p* I or 10μg *Der p* II in complete Freund's adjuvant (CFA). Mice were bled from the tail vein 14-21 days after the final injection and IgG antibodies in the sera were measured by antigen binding RIA using ^{125}I labelled Group I or Group II allergens.

ii) Comparison of antibody responses to *Der p* II in different BALB strains.

Groups of 3-5 mice (BALB/c, BALB.B, BALB.K, BALB.A10 or BALB.BD.2R) were immunized i.p. 3 times at 14 day intervals with 5μg *Der p* II in CFA, or at 15 day intervals with allergen adsorbed to 250μg aluminium hydroxide gel (Maalox^{R}, Rorer Inc, Fort Washington, PA). IgG antibody responses to *Der p* II or *Der f* II were determined by antigen binding RIA.

Mice were obtained from either Hilltop Laboratories, Scottdale, PA, or from Dr. David Sachs, Harvard University (BALB strains, currently available from Hazelton

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Laboratories, Rockville, MD). The use of animals for this study was approved by the Animal Research Committee of the University of Virginia, under guidelines for the use and care of animals formulated by the National Council for Medical Research.

Monoclonal antibodies to Group II allergens

Six A/J mice were immunized i.p. with 60μg *Der p* II in CFA. At two week intervals mice were boosted twice with 30μg *Der p* II. A final intrasplenic injection of 50μg *Der p* II was given to the mouse demonstrating the highest IgG antibody titer by mAb RIA (1/13,000). Three days later this mouse was sacrificed and its spleen cells were fused with SP_2/O myeloma cells using 37% polyethylene glycol, as described previously (23, 24). Two weeks post-fusion hybrids were screened by ELISA using a 50-80% SAS fraction of *D. pteronyssinus* extract (containing 1300μg/ml *Der p* II). Hybrids producing IgG anti-mite antibodies were tested for binding to ^{125}I *Der p* II and ^{125}I *Der f* II and cloned by limiting dilution. Positive clones were expanded and injected into pristane primed mice for ascites production. Nine anti-*Der p* II mAb were derived from this fusion. Three anti-*Der f* II mAb were also studied (clones 6D6, 7A1 and 4E5) and 4 mAb were kindly provided by either Dr. R. Aalberse (αDpX) or by Dr. H. Okudaira (13A4, 15E11 and 18G8) (20-22).

Antigen-binding RIA

This assay for measuring murine IgG antibody to mite allergens has been described previously (23, 24). Essentially, 100μl diluted mouse antiserum or mAb was incubated for 4hr with 100μl ^{125}I labeled allergen and IgG antibody-antigen complexes were precipitated by the addition of 100μl goat anti-mouse IgG. The precipitates were

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washed three times with BBS, pH 8.0 and counted in a gamma counter.

^{125}I labelling of proteins

Purified mAb or mite allergens were iodinated using a modified chloramine-T technique in which 20μg protein was labeled with 0.5mCi ^{125}I (Amersham Corp., Arlington Heights, IL) (25). The specific activity of ^{125}I *Der p* II or *Der f* II was 5-8μCi/μg protein, whilst that of the mAb or Group I allergens was 30-50μCi/μg.

Epitope analysis of mAb

The epitope specificity of mAb raised against *Der p* II was compared by solid-phase RIA (21, 24). Cyanogen bromide-activated paper discs were coupled with mAb (1μg/disc) and each disc was incubated for 2hr with 0.1ml of a 50-80% SAS fraction of *D. pteronyssinus* or *D. farinae* extract containing 1μg/ml *Der p* II or *Der f* II, respectively. Discs were washed five times with PBS-T and incubated with 500ng cold mAb together with ~5ng ^{125}I labeled mAb for 4hr. After washing, the discs were counted in a gamma counter. All assays were carried out in duplicate, and the sample diluent was 1% BSA PBS-T. Uninhibited binding of ^{125}I mAb to *Der p* II or *Der f* II was calculated from the mean cpm of 4 wells incubated with 1% BSA PBS-T. The percent inhibition of binding of ^{125}I mAb to *Der p* II by cold mAb was calculated as previously (24). Epitope specificity was also analyzed by two site binding RIA using a capture mAb to "present" *Der p* II to a second ^{125}I mAb. Sixteen solid phase mAb were each coupled to CNBr-activated paper discs and incubated for 4hr with 100μl of *D. pteronyssinus* or *D. farinae* extract. After washing, 5ng of each of six ^{125}I labelled mAb (100,000 cpm) was incubated with the discs for 2hr and the discs were washed and counted.

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Biotinylation of mAb

Anti-*Der p* II mAb, 1D8-C4 or 4G7-F1, were purified from ascites by HPLC on a 5ml recombinant protein G sepharose column (Gamma-Bind Plus, Ginex, Gaithersburg, MD) and dialyzed against 0.1M sodium bicarbonate, pH 8.4 overnight at 4°C. For biotinylation, 7.25mg mAb in a volume of 10.35ml (0.7mg/ml), was mixed with 1.24ml of Enzotin (EAB-40 G Enzobiochem, NY) in dimethyl sulfoxide for 4hr at room temperature (7). Enzotin was prepared at the same concentration as the mAb. Unreacted enzotin was removed by dialysis against 4L PBS at 4°C. Biotinylated mAb were titrated using *D. pteronyssinus* (UVA 92/01) and *D. farinae* (UVA 92/02) extracts to determine the optimal dilution for binding in the ELISA (this was 1/15,000). Biotinylated mAb were stored in the dark, at 4°C, diluted 1:15 in 50% glycerol/1% BSA PBS/0.01% thimerosal. Each aliquot contained 42μg/ml biotinylated mAb.

Measurement of Group II allergens by ELISA

The Group II ELISA was modified from the previously published assay for *Der p* I and *Der f* I (7). Plastic microtiter plates were coated overnight with 1μg/well of a 50% SAS cut of mAb 7A1 ascites in 0.05M bicarbonate buffer, pH 9.6 at 4°C. The plates were incubated with 1% BSA PBS-T for 1hr, washed, and incubated with dilutions of allergen or dust extracts. Bound allergen was detected by adding 100μl 1:1000 dilution of biotinylated mAb 1D8 (containing 4.2ng mAb) and, after further washing, 100μl 1:1000 dilution of streptavidin-peroxidase (Sigma, St Louis, MO). The enzyme substrate was 2,2'-azinobis-3-ethyl-benzthiazoline sulfonic acid and the green color reaction was read at 405nm (7).

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The ELISA was quantified using doubling dilutions of *D. pteronyssinus* or *D. farinae* extracts to form control curves from 0.5-250ng/ml. These extracts were prepared by Miles Laboratories, Spokane, WA, from whole mite bodies, and designated UVA 92/01 (*D. pteronyssinus*) and UVA 92/02 (*D. farinae*). Each extract had been substandardized against the Center for Biologics Evaluation and Research mite references, E1-Dp and E1-Df, which were estimated to contain 50μg/ml or 20μg/ml of *Der p* II or *Der f* II, respectively. Stock solutions of the UVA references were prepared at 5μg/ml in 50% glycerol/PBS/1% BSA/0.01% thimerosal and used at a 1:20 dilution to form the first dilution of the control curve (250ng/ml).

To validate the results of the ELISA 40 dust extracts from 20 houses in Sao Paulo, Brazil were assayed by ELISA and the results compared by linear regression analysis with these previously obtained by RIA (19).

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RESULTS

Murine IgG antibody responses to Group I and Group II allergens

Six inbred mouse strains were compared for IgG antibody responses to *Der p* I or *Der p* II (Table I). All responder mouse strains immunized with *Der p* I showed a predominantly species specific response, and in most cases IgG anti-*Der f* I antibody was <20% of the anti-*Der p* I antibody. These results are in keeping with previous data using polyclonal antibodies and mAb from BALB/c mice (23, 24, 26-28). AKR mice failed to make IgG antibodies to *Der p* I, even after prolonged immunization (5 injections of *Der p* I in CFA). In contrast to the response to *Der p* I, IgG antibody responses to *Der p* II were strongly cross-reactive with *Der f* II in all responder strains (Table I). BALB/c mice showed weak or undetectable responses to *Der p* II, which was unexpected, as the original anti-*Der p* II mAb, anti-DpX, had been raised from a BALB.B mouse (20). Antibody responses in congenic BALB strains were compared by immunizing mice with *Der p* II in either CFA or alum (Table II). The results showed that BALB.B, BALB.K, and BALB.A10 mice produced high levels of IgG anti-Group II antibody when immunized in CFA or Alum. BALB/c mice made weak antibody responses following immunization with CFA and made significantly lower titers than the other strains when immunized with *Der p* II in alum. Four BALB/c x BALB.B hybrid mice (BALB.BD.2R) were also immunized with *Der p* II in CFA, using the same immunization protocol, and these mice also had undetectable IgG antibodies to *Der p* II.

Specificity of mAb

A/J mice were used for anti-*Der p* II mAb production, because a BALB/c x A/J

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hybrid (CAF1) can be used for ascites production. Nine mAb were derived from a single fusion using spleen cells from an A/J mouse that had been immunized with *Der p* II in CFA. Binding of these mAb to radiolabeled *Der p* II or *Der f* II was compared with that of 7 other anti-Group II mAb derived from 3 previous fusions using BALB/B, BALB/c or A/J mice (Table III). The RIA results showed that most mAb (11/16) bound to cross-reactive epitopes on both *Der p* II and *Der f* II. Polyclonal mouse IgG anti-*Der p* II antibodies also bound equally well to both allergens. Three clones showed 5-10 fold greater binding to *Der p* II than *Der f* II (3G5-A2, 3G5-H11 and 2B12) and two clones showed significantly higher binding to *Der f* II (15E11 and 4E5). None of the mAb reacted with Group I allergens (data not shown).

Previous studies showed that mAb αDpX, 7A1 and 6D6 defined three non-overlapping cross-reactive epitopes on the Group II allergens (20, 21). The epitope specificity of the 16 mAb used in the present studies was compared by cross-inhibition RIA and by two site binding assay, using cold mab to present allergen to a second ^{125}I labelled mAb. For the cross-inhibition studies, cold mAb was used to inhibit the binding of ^{125}I labelled mAb (1D8 or 4G7) to allergen which was "presented" by a mAb coupled to a paper disc (either 7A1 or αDpX). When allergen was presented by mAb 7A1, the 1D8 and 4G7clones showed 70-98% cross-inhibition, suggesting that they were directed against the same epitope (Fig. 1A). In contrast, several other mAb (e.g. 4E5, 3G5 and 2B12) showed <30% inhibition and appeared to be directed against different epitopes. Similar results were obtained using mAb αDpX on the solid phase, though the levels of inhibition tended to be lower with this mAb (Fig. 1B). Most of the mAb raised in the

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present fusion appeared to have the same specificity as mAb 1D8 or 4G7 and this epitope appeared to be distinct from the three mAb defined sites reported previously (21).

Two site binding assays were carried out to confirm that the epitopes were non-overlapping and to identify mAb combinations that could be used in immunoassays. Six ^{125}I mAb were compared for binding to *Der p* II using each of the 16 mAb on the solid phase. Selected results are shown in Table IV. In each case, there was little or no binding when the solid phase mab was the same as the ^{125}I label. The mAb could be divided into two groups, based on binding of ^{125}I mAb. Some combinations, e.g. using mAb αDpX, 7A1 or 3G5 on the solid phase showed strong binding (20-50,000 cpm bound) using each ^{125}I mAb. Other mAb, e.g. 6D6, 1D8, 4G7 or 13A4, showed binding when used in combination with ^{125}I αDpX, but not with the other five ^{125}I mAb (Table IV). MAb αDpX could be used in a two site assay in combination with any of the other mAb, either on the solid phase or as ^{125}I detecting mAb, with the exception of mAb 15E11 which appeared to have similar specificity to αDpX. The two site binding assays were repeated using *Der f* II and similar patterns of reactivity were obtained (data not shown).

ELISA for Group II allergens

The original mAb RIA for Group II was carried out using anti-*Der f* II mAb 7A1 coupled to CNBr-activated cellulose discs and ^{125}I labeled anti-*Der p* II mAb αDpX for detection (21). Although several combinations of solid-phase mAb coupled to CNBr-activated discs were effective in two site RIA for the detection *Der p* II and *Der f* II, only 3/16 mAb (7A1, αDpX, 13A4) bound to plastic microtiter wells and could be used

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to develop an ELISA (see Table IV). These mAb were used in combination with biotinylated mAb 1D8 or 4G7 and ELISA control curves for either *Der p* II or *Der f* II were compared (Fig. 2A-D). Based on these curves, the combination of mAb 7A1 on the solid phase and biotinylated 1D8, was selected for routine use. This assay gives parallel control curves for both Group II allergens and is ~2 fold more sensitive to *Der f* II than *Der p* II (limits of sensitivity 2-4ng/ml, Fig. 3).

Forty house dust extracts from Sao Paulo, Brazil were analyzed for Group II allergens using both the mAb ELISA and mAb RIA. The results showed very good correlation between Group II levels obtained by ELISA and RIA ($n = 40$, $r = 0.85$, $p < 0.001$) (Fig. 4). The mean within assay coefficient of variation for two separate Group II assays was 12.1% ($n = 69$) and the mean inter assay coefficient of variation on two assays was 16.1% ($n = 15$). In addition, the correlation coefficient between ELISA and RIA for 11 mite extracts from different manufacturers was also very good ($r = 0.95$, $p < 0.001$) (data not shown).

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DISCUSSION

There have been few previous studies on murine immune responses to mite allergens. Studies on IgE antibody responses showed that CBA and C57Bl were high responders to *Der p* I, whereas BALB/c, C3H and AKR were poor responders (30). Most of the studies on IgG antibody responses have been confined to BALB/c mice because this is the preferred strain for mAb production (23, 24, 26-28). The present results show that all the mouse strains tested made high titer, species specific IgG antibody responses to *Der p* I with the exception of AKR mice, which were non-responders. Seven of the eight strains tested, including BALB congenics, also made antibody responses to *Der p* II, which were cross-reactive with *Der f* II. The poor antibody response of BALB/c (H-2^d) mice to Group II allergens has been previously reported and was confirmed by the present studies (21). In addition, BALB.BD.2R mice, which are also H-2^d at the I-A and I-E loci, did not make detectable anti-*Der p* II antibodies. These results are in keeping with a recent study of T cell responses to *Der p* II, in which H-2^d mice made poor T cell responses to *Der p* II, or *Der p* II peptides, whereas H-2^b mice were high responders and H-2^k mice were intermediate (31). In our studies, some H-2^k mice made high levels of IgG antibody to *Der p* II, comparable to those of H-2^b haplotype. Given both the antibody and T cell data, it is difficult to reconcile the production of three high affinity anti-*Der f* II mAb from BALB/c mice (22). The BALB/c derived mAb were produced following prolonged high dose immunization (7 x 30μg *Der f* II in Freund's adjuvant) and, in fact, the antibody and T cell studies each used different immunization protocols, suggesting that the route and dose of

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immunization can significantly affect immune responsiveness to *Der p* II. Analysis of the specificity of anti-Group II mAb produced as part of the present study, together with those produced previously, showed that the mAb define four non-overlapping epitopes, present on both *Der p* II and *Der f* II. These results are consistent with the high degree of amino acid sequence homology between the two allergens (88%, 32).

The development of an ELISA for Group II allergens has several potential applications in asthma and allergy research, including environmental studies, assessment of avoidance procedures and allergen standardization. Immunoassays for the Group I allergens are established as the primary method of assessing mite allergen exposure and threshold values have been proposed as risk factors for the induction of IgE antibody responses and for the exacerbation of asthma attacks (1-6). A good correlation between Group I and Group II allergen levels has been reported in house dust samples from Japan and Brazil, with an ~1:1 ratios of the two groups of allergens in dust extracts (9, 12, 19). The availability of the ELISA will make it possible to compare Group II allergen levels in other parts of the world and to investigate the epidemiologic significance of measuring Group I and Group II allergens. Measurements of both allergens would provide a better measure of "total" allergen load, and the Group II assay has the advantage that it measures allergen from both *D. pteronyssinus* and *D. farinae*.

The Group II ELISA will be particularly useful for mite avoidance studies and for allergen standardization. In terms of avoidance, the assay will be of practical value in assessing the effects of chemical treatments to reduce mite allergen levels, particularly treatments involving allergen denaturation, since it has been established that Group II

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allergens are more resistant to denaturation than Group I (33). Recent studies have shown that measurements of Group II were effective in monitoring the efficacy of benzyl benzoate and tannic acid, currently the two most commonly used acaricidal and chemical treatments (18, 34, 35). Group II measurements should play an important role in the standardization and quality control of mite extracts. The allergen content of mite extract varies significantly with the source material and it is now clear that extracts prepared from whole mite culture usually have a very high Group I;Group II ratio (often >10:1), whereas extracts of pure mite bodies usually have ratios of <2:1 (21). Measurements of either Group of allergens alone may not be representative of the composition or potency of the extract. Preliminary studies of mite body extracts marketed in the U.S., have shown that measurements of both Group I and Group II generally correlate with potency estimates based on skin testing (36). In those studies, it was also clear that some extracts had low major allergen content or had aberrant Group I:Group II ratios. Measurements of both allergens by ELISA provides a simple quantitative method of standardization which will also enable companies to improve their extraction and quality control procedures (37).

Our studies further emphasize the use and applications of mAb in research on mite and other indoor allergens, particularly for assessing environmental exposure. Thus although several other mite allergens have been identified, and in some cases fully sequenced, data on sensitization and exposure to these allergens is limited because few mAb to other allergens are available. The present studies suggest that the panel of mAb will provide useful probes for analyzing B cell epitopes on Group II allergens. The

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ELISA will be a useful adjunct for further epidemiologic studies of mite allergy, for developing improved diagnostic and therapeutic allergen extracts, and for monitoring the efficacy of allergen avoidance procedures, as part of the management of asthma.

Acknowledgements

We are very grateful to Dr. Rob Aalberse, University of Amsterdam, The Netherlands, and to Dr. Hiro Okudaira, Tokyo University, Japan for providing samples of their monoclonal antibodies for use in these studies. We thank Dr. William M. Sutherland and the staff of the University of Virginia Lymphocyte Culture Center for assistance with production monoclonal antibodies, and Dr. Karla Arruda for providing dust samples from Sao Paulo, Brazil. We thank Miles Laboratories for *D. pteronyssinus* and *D. farinae* culture. We are also grateful to Madeleine Watkins for drawing the figures and to Nancy Malone for secretarial support.

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LEGENDS TO FIGURES

Figure 1.

Cross-inhibition of the binding of ^{125}I labelled mAb to *Der p* II.

Solid phase mAb 7A1 (Panel A) and αDpX (Panel B) were used to present *Der p* II to 5 - 10ng ^{125}I labelled 4G7 (solid bars) or 1D8 (hatched bars). This binding was inhibited using 500ng cold αDpX (a), 6D6 (b), 7A1 (c), 4E5 (d), 4G7-F1 (e), 4G7-F2 (f), 1D8-C4 (g), 1D8-A6 (h), 3G5-H11 (i), 3G5-A2 (j), 1F9-C6 (k), 2B12-B3 (l), 1E12-E6 (m), 13A4 (n), 15E11 (o) or 18G8 (p). Total radioactivity added (in 0.1ml) = 108,839 cpm (4G7) and 111,840 cpm (1D8).

Figure 2.

ELISA curves for Group II mite allergens using different combinations of mAb.

The curves compare binding of *Der p* II (Panels A and C) or *Der f* II (Panels B and D) using mAb 13A4 (○), 7A1 (●) or αDpX (▲) as capture mAb, and biotinylated mAb 1D8 (Panels A and B) or 4G7 (Panels C and D) for detection.

Figure 3.

ELISA control curves for *Der p* II and *Der f* II using mAb 7A1 on the solid phase and biotinylated mAb 1D8 for detection.

Figure 4.

Correlation between the mAb ELISA and mAb RIA for *Der p* II in 40 house dust extracts collected from 20 houses in Sao Paulo, Brazil.

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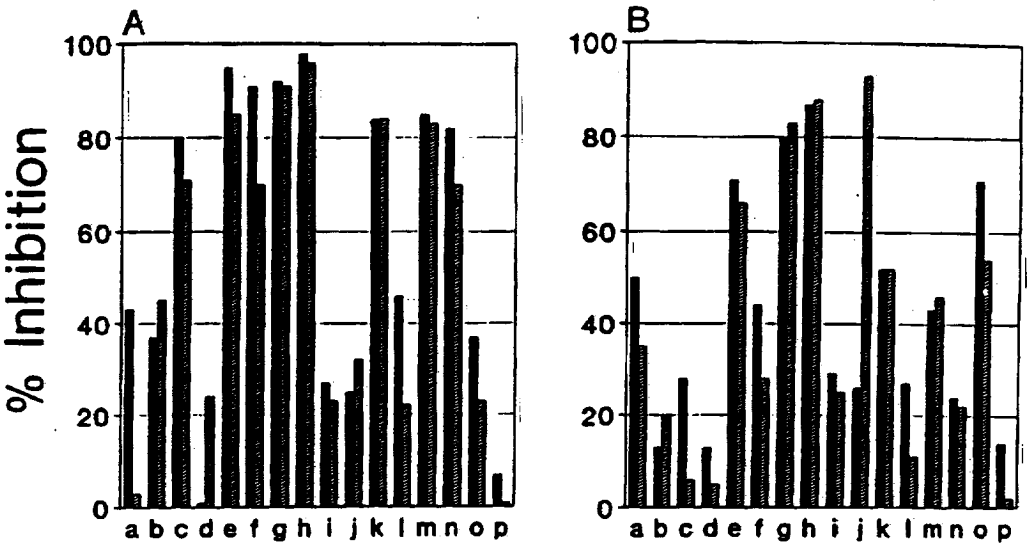

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Source: https://www.industrydocuments.ucsf.edu/docs/rknm0000

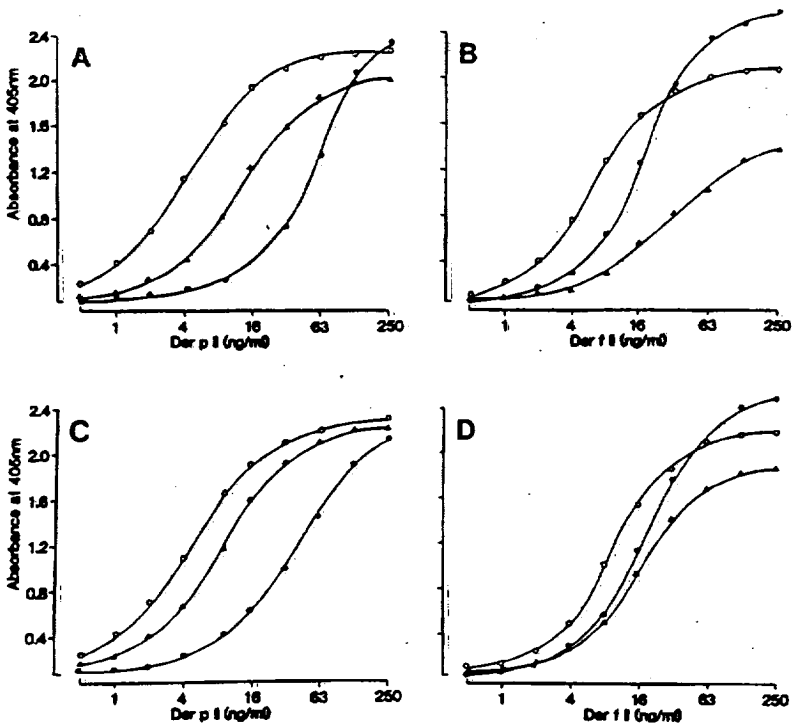

Figure 2.

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Source: https://www.industrydocuments.ucsf.edu/docs/rknm0000

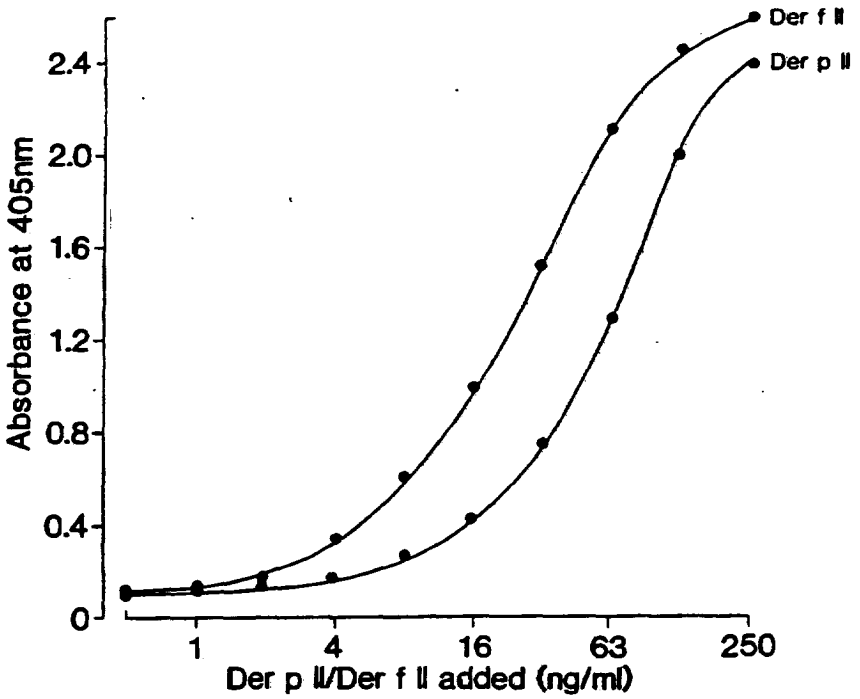

Figure 3.

ELISA control curves for *Der p* II and *Der f* II using mAb 7A1 on the solid phase and biotinylated mAb 1D8 for detection.

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Source: https://www.industrydocuments.ucsf.edu/docs/rknm0000

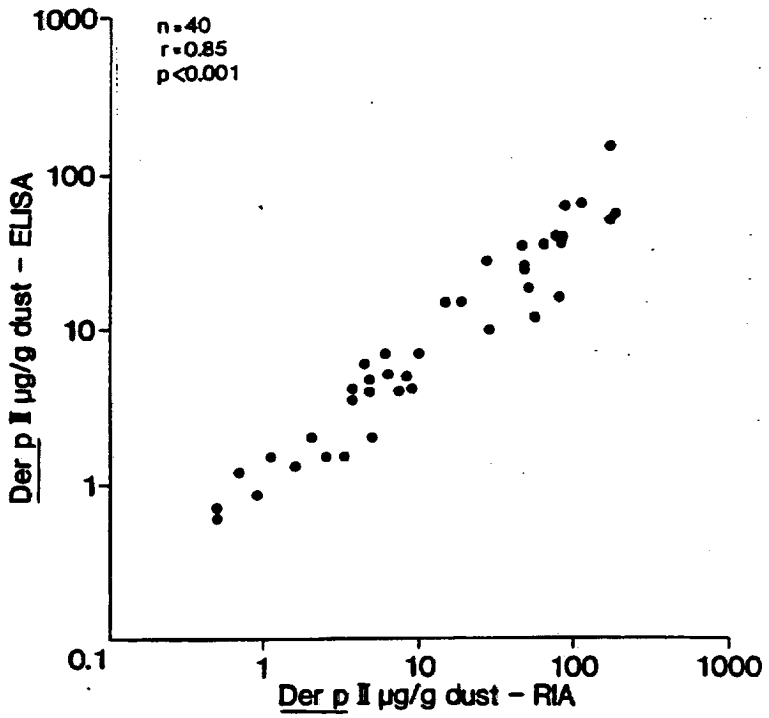

Figure 4.

Correlation between the mAb ELISA and mAb RIA for *Der p* II in 40 house dust extracts collected from 20 houses in Sao Paulo, Brazil.

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TABLE I

Specificity of polyclonal murine IgG ab responses to *Der p* I or *Der p* II*

Strain	H-2 type	μg allergen bound/ml serum*			
		Der p I	*Der f* I	*Der p* II	*Der f* II
BALB/c	d	27.2	1.5	<0.2	<0.2
C57BL6	b	15.0	0.5	11.1	7.7
C3H	k	3.3	0.4	2.9	5.0
CBA	k	10.9	2.1	12.7	16.2
A/J	a	3.5	2.0	3.1	5.0
AKR	k	<0.2	<0.2	n.d.	n.d.

* Groups of 5 mice were immunized 3x with 10μg allergen in CFA.

* Determined by antigen binding RIA using ^{125}I labelled allergens. Values represent the geometric mean of assay results on 5 sera.

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TABLE II

Polyclonal IgG anti-Group II ab responses in congenic BALB strains*

Strain	H-2 haplotype	Adjuvant	μg allergen bound/ml serum	
			Der p II	*Der f* II
BALB/c	d	CFA	0.23 ± 0.17	<0.2
		Alum	3.1 ± 0.7	0.5 ± 0.1
BALB.B	b	CFA	12.7 ± 2.4	3.2 ± 0.9
		Alum	18.9 ± 1.7	4.2 ± 0.6
BALB.K	k	CFA	17.7 ± 1.3	9.2 ± 3.5
		Alum	17.4 ± 1.6	4.0 ± 0.3
BALB.A10	a.10	CFA	15.7 ± 1.5	2.7 ± 0.5
		Alum	12.6 ± 1.7	3.2 ± 0.5
BALB.BD(2R)	g	CFA	<0.2	<0.2

* Groups of 3-5 mice were immunized 3x with 5μg *Der p* II in either CFA or alum. Sera were assayed for IgG antibody by antigen binding assay and the results are expressed as the geometric mean ±SEM.

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TABLE III

Monoclonal Antibodies to Dermatophagoides Group II Allergens

mAb ¤	Immunogen	Mouse strain	^{125}I allergen bound (cpm) #	
			Der p II	*Der f* II
αDpX *	*Der p* II	BALB.B	18,029	11,143
6D6 §	*Der f* II	A/J	5,104	12,772
7A1 §	*Der f* II	A/J	9,028	11,756
4E5 §	*Der f* II	A/J	1,617	22,659
4G7-F1	*Der p* II	A/J	10,738	13,778
4G7-F2	*Der p* II	A/J	12,678	6,336
1D8-C4	*Der p* II	A/J	10,775	16,796
1D8-A6	*Der p* II	A/J	10,423	14,924
3G5-H11	*Der p* II	A/J	10,674	1,105
3G5-A2	*Der p* II	A/J	7,720	808
1F9-C6	*Der p* II	A/J	11,170	5,118
2B12-B3	*Der p* II	A/J	3,024	807
1E12-E6	*Der p* II	A/J	13,946	5,493
15E11 +	*Der f* II	BALB/c	3,672	21,422
13A4 +	*Der f* II	BALB/c	5,682	17,346
18G8 +	*Der f* II	BALB/c	1,336	2,284
Controls:				
6F9 **	*Fel d* I	BALB/c	1,344	872
Normal mouse serum		-	1,317	815
Polyclonal mouse anti-serum	*Der p* II	CBA	11,830	15,445

¤ All clones were of the IgG1 isotype, with the exception of 2B12, which was IgG2b.

By antigen binding RIA using mAb ascites at 1/2500 dilution. MAb provided by Dr. R. Aalberse (*), and Dr. H. Okudaira (+), or obtained in a previous study (§, 21).

** Control mAb raised against cat allergen *Fel d* I, (29).

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TABLE IV

Comparison of mAb binding to *Der p* II by two site RIA[#]

Solid phase mAb	Immunogen	^{125}I mAb added: αDpX	6D6	4G7	1D8	2B12	1E12
αDpX	*Der p* II	2,109	36,897	40,853 +	36,572 +	22,058	21,108
6D6	*Der f* II	32,320	793	1,662	2,120	1,731	3,121
7A1	*Der f* II	50,728	52,913	45,599 +	41,964 +	30,156	18,603
4E5	*Der f* II	11,869	20,777	23,987	26,100	20,555	9,416
1D8-C4*	*Der p* II	33,494	555	2,271	2,225	2,133	3,043
4G7-F1	*Der p* II	33,256	592	3,315	2,433	2,238	2,488
3G5-H11	*Der p* II	35,047	42,031	30,288	33,408	23,542	15,042
2B12	*Der p* II	24,748	513	1,186	1,490	1,642	2,460
15E11	*Der f* II	1,470	23,995	30,326	22,576	22,716	15,355
13A4	*Der f* II	39,552	48,713	34,774 +	37,512 +	22,663	2,002
PBS control§		444±123	408±69	877±145	855±145	1,154±201	2,268±365

Solid phase mAb were coupled to CNBr-activated paper discs (1 μg/disc). Discs were incubated with *D. pteronyssinus* extract (1μg/ml) overnight, washed and incubated with different ^{125}I second mAb (~110,000 cpm).

* MAb with the same epitope specificity to *Der p* II were grouped and clones with the similar specificity as the 1D8-C4 were as follows: 1D8-A6, 1E12-E6, 1F9-C6, 4G7-F1, 4G7-F2.

§ PBS control results are expressed as the mean cpm ± s.d. for each group of mAb tested.

\+ These combinations of mAb could be used in RIA on plastic microtiter wells and were used to develop an ELISA. The other combinations only showed significant binding in the paper disc assay.

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